REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Large-scale multiplex analysis of highly polymorphic loci is needed for practical identification of individuals, e.g., for paternity testing and in forensic science, for organ-transplant donor-recipient matching, for genetic disease diagnosis, prognosis, and pre-natal counseling, and the study of oncogenic mutations. In addition, the cost-effectiveness of infectious disease diagnosis by nucleic acid analysis varies directly with the multiplex scale in panel testing. Many of these applications depend on the discrimination of single-base differences at a multiplicity of sometimes closely space loci.

A variety of DNA hybridization techniques are available for detecting the presence of one or more selected polynucleotide sequences in a sample containing a large number of sequence regions. In a simple method, which relies on fragment capture and labeling, a fragment containing a selected sequence is captured by hybridization to an immobilized probe. The captured fragment can be labeled by hybridization to a second probe which contains a detectable reporter moiety.

Another widely used method is Southern blotting. In this method, a mixture of DNA fragments in a sample are fractionated by gel electrophoresis, then fixed on a nitrocellulose filter. By reacting the filter with one or more labeled probes under hybridization conditions, the presence of bands containing the probe sequence can be identified. The method is especially useful for identifying fragments in a restriction-enzyme DNA digest which contain a given probe sequence, and for analyzing restriction-fragment length polymorphisms ("RFLPs").

Another approach to detecting the presence of a given sequence or sequences in a polynucleotide sample involves selective amplification of the sequence(s) by polymerase chain reaction. In this method, primers complementary to opposite end portions of the selected sequence(s) are used to promote, in conjunction with thermal cycling, successive rounds of primer-initiated replication. The amplified sequence may be readily identified by a variety of techniques. This approach is particularly useful for detecting the presence of low-copy sequences in a polynucleotide-containing sample, e.g., for detecting pathogen sequences in a body-fluid sample.

More recently, methods of identifying known target sequences by probe ligation methods have been reported. In one approach, known as oligonucleotide ligation assay ("OLA"), two probes or probe elements which span a target region of interest are hybridized with the target region. Where the probe elements match (basepair with) adjacent target bases at the confronting ends of the probe elements, the two elements can be joined by ligation, e.g., by treatment with ligase. The ligated probe element is then assayed, evidencing the presence of the target sequence.

In a modification of this approach, the ligated probe elements act as a template for a pair of complementary probe elements. With continued cycles of denaturation, hybridization, and ligation in the presence of the two complementary pairs of probe elements, the target sequence is amplified geometrically, i.e., exponentially allowing very small amounts of target sequence to be detected and/or amplified. This approach is referred to as ligase chain reaction ("LCR").

Another scheme for multiplex detection of nucleic acid sequence differences is disclosed in U.S. Patent No. 5,470,705 to Grossman et. al. where sequence-specific probes, having a detectable label and a distinctive ratio of charge/translational frictional drag, can be hybridized to a target and ligated together. This technique was used in Grossman, et. al., "High-density Multiplex Detection of Nucleic Acid Sequences: Oligonucleotide Ligation Assay and Sequence-coded Separation," Nucl. Acids Res. 22(21):4527-34 (1994) for the large scale multiplex analysis of the cystic fibrosis transmembrane regulator gene.

Jou, et. al., "Deletion Detection in Dystrophin Gene by Multiplex Gap Ligase Chain Reaction and Immunochromatographic Strip Technology," Human Mutation 5:86-93 (1995) relates to the use of a so called "gap ligase chain reaction" process to amplify simultaneously selected regions of multiple exons with the amplified products being read on an immunochromatographic strip having antibodies specific to the different haptens on the probes for each exon.

There is a growing need, e.g., in the field of genetic screening, for methods useful in detecting the presence or absence of each of a large number of sequences in a target polynucleotide. For example, as many as 400 different mutations have been associated with cystic fibrosis. In screening for genetic predisposition to this disease, it is optimal to test all of the possible different gene sequence mutations in the subject's genomic DNA, in order to make a positive identification of "cystic fibrosis". It would be ideal to test for the presence or

absence of all of the possible mutation sites in a single assay. However, the prior-art methods described above are not readily adaptable for use in detecting multiple selected sequences in a convenient, automated single-assay format.

Solid-phase hybridization assays require multiple liquid-handling steps, and some incubation and wash temperatures must be carefully controlled to keep the stringency needed for single-nucleotide mismatch discrimination. Multiplexing of this approach has proven difficult as optimal hybridization conditions vary greatly among probe sequences.

Allele-specific PCR products generally have the same size, and a given amplification tube is scored by the presence or absence of the product band in the gel lane associated with each reaction tube. This approach requires splitting the test sample among multiple reaction tubes with different primer combinations, multiplying assay cost. PCR has also discriminated alleles by attaching different fluorescent dyes to competing allelic primers in a single reaction tube, but this route to multiplex analysis is limited in scale by the relatively few dyes which can be spectrally resolved in an economical manner with existing instrumentation and dye chemistry. The incorporation of bases modified with bulky side chains can be used to differentiate allelic PCR products by their electrophoretic mobility, but this method is limited by the successful incorporation of these modified bases by polymerase, and by the ability of electrophoresis to resolve relatively large PCR products which differ in size by only one of these groups. Each PCR product is used to look for only a single mutation, making multiplexing difficult.

Ligation of allele-specific probes generally has used solid-phase capture or size-dependent separation to resolve the allelic signals, the latter method being limited in multiplex scale by the narrow size range of ligation probes. The gap ligase chain reaction process requires an additional step -- polymerase extension. The use of probes with distinctive ratios of charge/translational frictional drag technique to a more complex multiplex will either require longer electrophoresis times or the use of an alternate form of detection.

The need thus remains for a rapid single assay format to detect the presence or absence of multiple selected sequences in a polynucleotide sample.

Ordered arrays of oligonucleotides immobilized on a solid support have been proposed for sequencing, sorting, isolating, and manipulating DNA. It has been recognized that hybridization of a cloned single-stranded DNA molecule to all possible oligonucleotide

probes of a given length can theoretically identify the corresponding complementary DNA segments present in the molecule. In such an array, each oligonucleotide probe is immobilized on a solid support at a different predetermined position. All the oligonucleotide segments in a DNA molecule can be surveyed with such an array.

One example of a procedure for sequencing DNA molecules using arrays of oligonucleotides is disclosed in U.S. Patent No. 5,202,231 to Drmanac, et. al. This involves application of target DNA to a solid support to which a plurality of oligonucleotides are attached. Sequences are read by hybridization of segments of the target DNA to the oligonucleotides and assembly of overlapping segments of hybridized oligonucleotides. The array utilizes all possible oligonucleotides of a certain length between 11 and 20 nucleotides, but there is little information about how this array is constructed.

WO 89/10977 to Southern discloses the use of a support carrying an array of oligonucleotides capable of undergoing a hybridization reaction for use in analyzing a nucleic acid sample for known point mutations, genomic fingerprinting, linkage analysis, and sequence determination. The matrix is formed by laying nucleotide bases in a selected pattern on the support. This reference indicates that a hydroxyl linker group can be applied to the support with the oligonucleotides being assembled by a pen plotter or by masking.

WO 94/11530 to Cantor also relates to the use of an oligonucleotide array to carry out a process of sequencing by hybridization. The oligonucleotides are duplexes having overhanging ends to which target nucleic acids bind and are then ligated to the non-overhanging portion of the duplex. The array is constructed by using streptavidin-coated filter paper which captures biotinylated oligonucleotides assembled before attachment.

WO 93/17126 to Chetverin uses sectioned, binary oligonucleotide arrays to sort and survey nucleic acids. These arrays have a constant nucleotide sequence attached to an adjacent variable nucleotide sequence, both bound to a solid support by a covalent linking moiety. The constant nucleotide sequence has a priming region to permit amplification by PCR of hybridized strands. Sorting is then carried out by hybridization to the variable region. Sequencing, isolating, sorting, and manipulating fragmented nucleic acids on these binary arrays are also disclosed. In one embodiment with enhanced sensitivity, the immobilized oligonucleotide has a shorter complementary region hybridized to it, leaving part of the oligonucleotide uncovered. The array is then subjected to hybridization conditions so that a complementary nucleic acid anneals to the immobilized oligonucleotide. DNA ligase is then

used to join the shorter complementary region and the complementary nucleic acid on the array. There is little disclosure of how to prepare the arrays of oligonucleotides.

WO 92/10588 to Fodor et. al., discloses a process for sequencing, fingerprinting, and mapping nucleic acids by hybridization to an array of oligonucleotides. The array of oligonucleotides is prepared by a very large scale immobilized polymer synthesis which permits the synthesis of large, different oligonucleotides. In this procedure, the substrate surface is functionalized and provided with a linker group by which oligonucleotides are assembled on the substrate. The regions where oligonucleotides are attached have protective groups (on the substrate or individual nucleotide subunits) which are selectively activated. Generally, this involves imaging the array with light using a mask of varying configuration so that areas exposed are deprotected. Areas which have been deprotected undergo a chemical reaction with a protected nucleotide to extend the oligonucleotide sequence where imaged. A binary masking strategy can be used to build two or more arrays at a given time. Detection involves positional localization of the region where hybridization has taken place. K. L. Beattie, et. al., "Advances in Genosensor Research," Clin. Chem. 41(5): 700-09 (1995) discloses attachment of previously assembled oligonucleotide probes to a solid support.

There are many drawbacks to the procedures for sequencing by hybridization to such arrays. Firstly, a very large number of oligonucleotides must be synthesized. Secondly, there is poor discrimination between correctly hybridized, properly matched duplexes and those which are mismatched. Finally, certain oligonucleotides will be difficult to hybridize to under standard conditions, with such oligonucleotides being capable of identification only through extensive hybridization studies.

The present invention is directed toward overcoming these deficiencies in the art.

Support for the amendments to claim 89 is found, for example, at page 49, lines 16-18 and page 35, lines 12-21 of the specification.

Applicants acknowledge the acceptance by the U.S. Patent and Trademark Office ("PTO") of informal drawings for examination purposes.

The PTO has indicated that the oath or declaration in the present application is defective for non-initialed changes. In response to this objection, applicants have submitted herewith a supplemental oath or declaration.

The rejection of claims 89-97, 109, and 111-112 under 35 U.S.C. § 112, second paragraph, for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 89-97, 109, and 111-112 under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,510,270 to Fodor et al. ("Fodor I") is respectfully traversed.

Initially, applicants note that Fodor I is not available as prior art under 35 U.S.C. § 102(b). In particular, the present application is a divisional of U.S. Patent Application Serial No. 08/794,851 ("'851 application), filed February 4, 1997, which claims the benefit of U.S. Provisional Patent Application Serial No. 60/01,359 ("'359 application), filed February 9, 1996. As Fodor I issued on April 23, 1996, less than one year prior to the filing date of the '851 application and after the filing date of the '359 application, at most, Fodor I is prior art under 35 U.S.C. § 102(e).

Fodor I relates to a method for synthesizing and screening polymers on a solid substrate. The method includes providing a substrate which may include linker molecules on its surface. On the substrate or a distal end of the linker molecules, a functional group with a protective group is provided. The protective group may be removed upon exposure to radiation, electric fields, electric currents, or other activators to expose the functional group. Using photolithographic methods, the protective group is removed in first selected regions and the substrate is contacted with a first monomer which reacts with the functional group. Thereafter, second selected regions are deprotected and exposed to a second monomer which reacts with the exposed functional group. These steps are repeated until the substrate includes desired polymers of desired lengths. Monomers may include amino acids, nucleotides, pentoses, and hexoses.

In contrast, amended claim 89 (and its dependent claims 90-119) relates to "A method of forming arrays of oligonucleotides on a solid support comprising: providing a solid support having an array of positions each suitable for attachments of an oligonucleotide; attaching to the solid support a surface or linker, suitable for coupling an oligonucleotide to the solid support, at each of the array positions; forming an array of a plurality of capture oligonucleotides on the solid support by a series of cycles of activating selected array positions for attachment of multimer nucleotides and attaching multimer nucleotides at activated array positions; and selecting the capture oligonucleotides to hybridize with

complementary oligonucleotide target sequences under hybridization conditions such that said capture oligonucleotides bind to complementary oligonucleotide targets with a similar hybridization stability across the array." Fodor neither discloses nor suggests selecting the capture oligonucleotides, such that they bind to complementary oligonucleotide targets with a similar hybridization stability across the array, as required by the claims of the present application. Accordingly, the rejection based on Fodor is improper and should be withdrawn.

The rejection of claims 89-94, 96-97, 109, and 111-112 under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,527,681 to Holmes ("Holmes") is respectfully traversed.

Initially, applicants note that Holmes is not available as prior art under 35 U.S.C. § 102(b). As described above, the present application is a divisional of U.S. Patent Application Serial No. 08/794,851 ("'851 application), filed February 4, 1997, which claims the benefit of U.S. Provisional Patent Application Serial No. 60/01,359 ("'359 application), filed February 9, 1996. As Holmes issued on June 18, 1996, less than one year prior to the filing date of the '851 application and after the filing date of the '359 application, at most, Holmes is prior art under 35 U.S.C. § 102(e).

Holmes relates to methods, devices, and compositions for synthesis and use of diverse molecular sequences on a substrate. In particular, this reference discloses the synthesis of an array of polymers in which individual monomers in a lead polymer are systematically substituted with monomers from one or more basis sets of monomers. On the substrate or a distal end of linker molecules, a functional group with a protective group is provided. The protective group may be removed upon exposure to a chemical reagent, radiation, electric fields, electric currents, or other activators to expose the functional group. Using photolithographic methods, the protective group is removed in first selected regions and the substrate is contacted with a first monomer which reacts with the functional group. Thereafter, second selected regions are deprotected and exposed to a second monomer which reacts with the exposed functional group. These steps are repeated until the substrate includes desired polymers of desired lengths. Monomers may include amino acids, nucleotides, pentoses, and hexoses.

In contrast, amended claim 89 (and its dependent claims 90-119) relates to "A method of forming arrays of oligonucleotides on a solid support comprising: providing a solid support having an array of positions each suitable for attachments of an oligonucleotide;

attaching to the solid support a surface or linker, suitable for coupling an oligonucleotide to the solid support, at each of the array positions; forming an array of a plurality of capture oligonucleotides on the solid support by a series of cycles of activating selected array positions for attachment of multimer nucleotides and attaching multimer nucleotides at activated array positions; and selecting the capture oligonucleotides to hybridize with complementary oligonucleotide target sequences under hybridization conditions such that said capture oligonucleotides bind to complementary oligonucleotide targets with a similar hybridization stability across the array." Holmes neither discloses nor suggests selecting the capture oligonucleotides, such that they bind to complementary oligonucleotide targets with a similar hybridization stability across the array, as required by the claims of the present application. Accordingly, the rejection based on Holmes is improper and should be withdrawn.

The rejection of claims 89 and 93 under 35 U.S.C. § 102(b) as being anticipated by Lipshutz et al., Biotechniques 19(3):442-447 (1995) ("Lipshutz") is respectfully traversed.

Lipshutz relates to a method of light-directed chemical synthesis to create high-density arrays of oligonucleotide probes, which can be used for detection of hybridized targets. In particular, this reference discloses the synthesis of an array of oligonucleotides in which linker molecules with a photochemically removable protective group are attached to a solid substrate. Light is directed through a photolithographic mask to specific areas of the surface, activating those areas for chemical coupling. The first of a series of nucleosides is incubated with the array, and chemical coupling occurs at those sites that have been illuminated in the preceding step. Next, light is directed to a different region of the substrate through a new mask, and the chemical cycle is repeated. The process is repeated.

In contrast, claim 89 (and its dependent claims 90-119) is directed to "[a] method of forming arrays of oligonucleotides on a solid support comprising: providing a solid support having an array of positions each suitable for attachments of an oligonucleotide; attaching to the solid support a surface or linker, suitable for coupling an oligonucleotide to the solid support, at each of the array positions; forming an array of a plurality of capture oligonucleotides on the solid support by a series of cycles of activating selected array positions for attachment of multimer nucleotides and attaching multimer nucleotides at activated array positions; and selecting the capture oligonucleotides to hybridize with

complementary oligonucleotide target sequences under hybridization conditions such that said capture oligonucleotides bind to complementary oligonucleotide targets with a similar hybridization stability across the array" (emphasis added). Lipshutz neither discloses nor suggests attaching multimer nucleotides at activated array positions, as required by claim 89 and its dependent claims. In contrast, Lipshutz teaches attaching single nucleosides to sites that have been illuminated in the preceding step. By serially attaching an oligonucleotide, as in Lipshutz, an array of 1,000 addresses would require over 40 days of around-the-clock work to prepare (see Specification at page 34, lines 19-21). Arrays containing short oligonucleotides of 8- to 10-mers do not have commercial applicability, because longer molecules are needed to detect single base differences effectively (see Specification at page 34, lines 21-23).

In contrast, attaching multimer nucleotides, such as tetramers, in accordance with the present invention, allows construction of 24-mers at any given address in only six steps with a likely improvement in overall yield by comparison to stepwise synthesis (see Specification at page 41, lines 6-8). This approach eliminates the presence of failure sequences on the support, which could occur when monomers are added one-at-a-time to the surface (see Specification at page 41, lines 8-9). Hence, in contrast to prior technologies, such as Lipshutz, the possibilities for false signals are reduced (see Specification at page 41, line 10). Moreover, since failure sequences at each address are shorter and lacking at least four bases (in the present example), there is no risk that these will interfere with correct hybridization or lead to incorrect hybridizations (see Specification at page 41, lines 11-13). This also means that "capping" steps will not be necessary (see Specification at page 41, lines 13-14).

Moreover, Lipshutz neither discloses nor suggests selecting the capture oligonucleotides, such that they bind to complementary oligonucleotide targets with a similar hybridization stability across the array, as required by the claims of the present application. In contrast, Lipshutz identifies the generation of signal from GC-rich and AT-rich probes (which have different hybridization strengths) in the same experiment as an "important challenge" to be met for broad implementation of the disclosed method (see page 47, col. 1, lines 21-30). However, Lipshutz neither discloses nor suggests any techniques for achieving binding of capture oligonucleotides to complementary oligonucleotide targets with a similar

hybridization stability across the array. Accordingly, the rejection based on Lipshutz is improper and should be withdrawn.

The rejection of claims 89 and 93 under 35 U.S.C. § 102(b) as being anticipated by Fodor et al., Nature 364:555-556 (1993) ("Fodor II") is respectfully traversed.

Fodor II relates to the synthesis of biological arrays by attaching linkers modified with photochemically removable protecting groups to a solid substrate. Light is directed through a photolithographic mask to specific areas of the surface effecting localized photodeprotection. The first of a series of chemical building bocks (e.g., hydroxyl photoprotected deoxynucleosides) is incubated with the surface, and chemical coupling occurs at those sites which have been illuminated in the preceding step. Next, light is directed to a different region of the substrate through a new mask, and the chemical cycle is repeated.

In contrast, claim 89 (and its dependent claims 90-119) is directed to "[a] method of forming arrays of oligonucleotides on a solid support comprising: providing a solid support having an array of positions each suitable for attachments of an oligonucleotide; attaching to the solid support a surface or linker, suitable for coupling an oligonucleotide to the solid support, at each of the array positions; forming an array of a plurality of capture oligonucleotides on the solid support by a series of cycles of activating selected array positions for attachment of multimer nucleotides and attaching multimer nucleotides at activated array positions; and selecting the capture oligonucleotides to hybridize with complementary oligonucleotide target sequences under hybridization conditions such that said capture oligonucleotides bind to complementary oligonucleotide targets with a similar hybridization stability across the array" (emphasis added). Fodor II neither discloses nor suggests attaching multimer nucleotides at activated array positions, as required by claim 89 and its dependent claims. In contrast, Fodor II teaches attaching single nucleosides to sites that have been illuminated in the preceding step.

As described above, attaching multimer nucleotides, such as tetramers, in accordance with the present invention, allows construction of 24-mers at any given address in only six steps with a likely improvement in overall yield by comparison to stepwise synthesis (see Specification at page 41, lines 6-8). This approach eliminates the presence of failure sequences on the support, which could occur when monomers are added one-at-a-time to the surface (see Specification at page 41, lines 8-9). Hence, in contrast to prior technologies,

such as Fodor II, the possibilities for false signals are reduced (see Specification at page 41, line 10). Moreover, since failure sequences at each address are shorter and lacking at least four bases (in the present example), there is no risk that these will interfere with correct hybridization or lead to incorrect hybridizations (see Specification at page 41, lines 11-13). This also means that "capping" steps will not be necessary (see Specification at page 41, lines 13-14).

Moreover, Fodor II neither discloses nor suggests selecting the capture oligonucleotides, such that they bind to complementary oligonucleotide targets with a similar hybridization stability across the array, as required by the claims of the present application. Accordingly, the rejection based on Fodor II is improper and should be withdrawn.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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